BACTERICIDAL ACTION OF ULTRAVIOLET RADIATION ON AIR-BORNE ORGANISMS

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Received for publication July 29, 1941

In the study of the bactericidal action of ultraviolet radiation on air-borne organisms, it is difficult to determine the number of bacteria per unit volume floating in the air and to measure the lethal radiation to which the organisms were exposed. In studying this action, Fair and Wells (1935) atomized a water suspension of *Escherichia coli* into a room and measured the normal rate at which the bacteria disappeared by sampling the air at regular intervals using a Wells air centrifuge. The process was then repeated, except that between the first and second samplings the air was irradiated for a short time with ultraviolet light. The lethal action due to the radiation superimposed upon the normal die-away was thus determined. From the results obtained, the conclusion was reached that "In relatively dry air the lethal power of ultraviolet light is from ten to one hundred times greater than is found when organisms are exposed on the surfaces of agar plates or are irradiated in water or other denser media."

To permit a better control of air motion thereby making possible more accurate measurements, Whisler (1936) atomized the bacteria into air drawn through a specially constructed duct. The air-borne bacteria passing along the duct were exposed as they approached an ultraviolet light source. The air was sampled at different places along the duct with an air centrifuge. In analyzing the action of the ultraviolet upon the bacteria it was assumed that "the quantum theory of light applies to the bactericidal action of ultraviolet radiation; that a single photon in the lethal wave band absorbed by a vital part of a bacterium is sufficient to kill it; and that all organisms of one species are identical." Based upon these assumptions and the results of the air samples taken along the duct, Wells (1940) concludes that "an organism suspended in dry air is perhaps twenty times as vulnerable to a given wave length as when suspended in water."

Whisler (1936) (1940), Wells and Wells (1936), and Koller (1939) find that air-borne bacteria are about ten times as resistant to radiation at high relative humidity as when floating in air at low humidity. Whisler attributes this to a "physical rather than a biological factor." According to Koller "It seems more reasonable to think that the effect is a physiological one."

Theoretically it is important to determine whether there is a greater action of ultraviolet on air-borne bacteria than on the same organisms seeded on agar, and whether there is any effect due to humidity. In the practical use of ultraviolet radiation as a bactericidal agent for air sterilization this information is essential.

EXPERIMENTAL

In an earlier paper by the authors (1941) it was shown that a bacterium is not equally resistant to ultraviolet radiation throughout its life span, and that in a bacterial culture the different individual organisms are never all at the same physiological stage. To compare the effects of ultraviolet radiation on bacteria floating in air with the vulnerability of organisms seeded on agar, it is therefore necessary that the distribution of the physiological stages of the different bacteria in a test be the same. This condition was approached by exposing a portion of the organisms of a culture, while floating in air, to a fixed amount of radiation, while an identically similar portion was seeded on the agar of a Petri plate and then exposed to the same amount of radiation. This was accomplished in the following manner: To the bottom of the spray box described in the previous

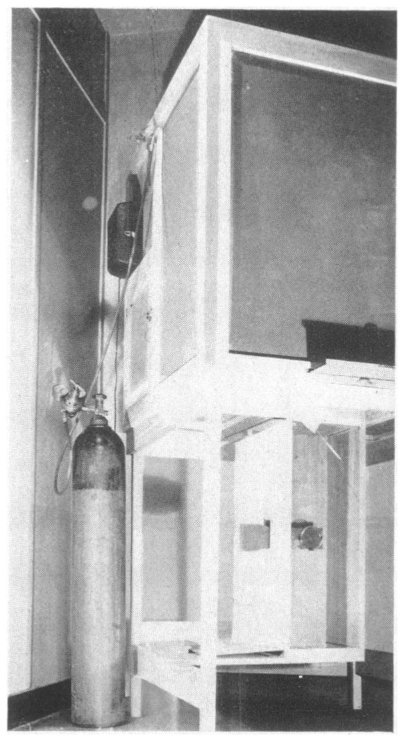

FIG. 1. SPRAY BOX AND TOWERS

paper was attached a double tower with a slider at the top and a second one near the bottom of the towers (fig. 1). The slider at the top was used to regulate the entrance of floating bacteria from the box to the towers. The slider at the bottom served to keep the bacteria in the respective towers from settling on sterile agar Petri plates in the trays on the floors of the towers until the slider was withdrawn. One side wall of one of the towers was made of ultraviolet transmitting glass through which the floating bacteria were exposed to radiation. The lamps used to produce the ultraviolet were placed at an appreciable distance from the glass window so that the intensity of the radiation in the tower was practically uniform throughout. The tantalum photocell of the meter described in the earlier paper (1941) was placed in a fixed position with respect to the lamps and its reading determined in terms of the average intensity inside the tower.

In making a test on the effect of radiation on air-borne bacteria, the slider between the spray box and the towers was closed and eight sterile agar plates were placed in the closed trays at the bottom, four in each of the towers. A suitable concentration of *E. coli* in broth was then sprayed into the box. The spray was allowed to settle for as much as 20 to 30 minutes to permit large drops to precipitate and turbulence to subside. The slider between the box and the towers was then opened for one minute thus admitting to the two towers the same number and the same general distribution of floating organisms. The top slider was then closed and the floating bacteria in one tower exposed to a definite amount of radiation as recorded by the meter. All the organisms that remained in suspension when the lamps were again turned off were floating during the entire exposure and consequently recived the same amount of radiation. The bottom slider over the trays containing the Petri plates was then withdrawn, and the floating bacteria allowed to collect on the plates for one hour. The plates in the two towers were thus seeded with identical organisms except that some in the irradiated tower had been killed by ultraviolet radiation during the exposure. Two of the four plates from the control tower were then placed at the center of the exposure tower facing the lights and exposed to the same amount of radiation, as measured by the meter, as had been given the floating bacteria. The two plates from the control towers that were not exposed were used as controls. All the plates were then incubated and the surviving colonies counted. From the reduction in the number of colonies on the plates exposed after seeding, and on the plates in the irradiated tower, the per cent killed by the radiation was calculated. Check tests using different seeding and settling times and different exposures and humidities were made as shown in table 1. Within experimental error the two percentages in any one test were the same. These results indicate that a bacterium floating in air at high or at low relative humidity requires the same amount of radiation as is needed to kill the same bacterium when seeded on the surface of agar in a Petri plate. Clearly the results obtained by Whisler with the duct tests do not agree with the tower experiments and, in one or the other, some variable was overlooked.

Whisler and Wells in calculating their results assumed that the survival number of bacteria, N, at time, t, when subjected to ultraviolet radiation of intensity, i, is an exponential function of time of the form

$$N = N_0 e^{-kit} \tag{1}$$

where, N_0, is the original number when the radiation was first applied, k, is a constant depending upon the organism and the character of the radiation. The per cent killed is expressed by the equation

$$\frac{N_0 - N}{N_0} = 1 - e^{-Kit} \tag{2}$$

If all the individual bacteria in a pure strain are identical, as was assumed in the duct experiments, k, is a constant, and the shape of the curve expressing the relation of the per cent killed to the ultraviolet exposure, is not altered by first exposing a number of simultaneously seeded plates to a constant amount of one type of radiation (as to Grenz rays or x-rays) followed by the action of ultraviolet light.

TABLE 1

Comparison of the percentage of E. coli killed by an equal amount of ultraviolet radiation on air-borne bacteria and similar bacteria seeded on Petri plates

The amount of radiation varies between tests; the relative humidities for the different tests are as shown in the table.

RELATIVE HUMIDITY	PER CENT KILLED	
	Floating	Fixed on plate
per cent		
37	33	24
	51	29
	30	27
	63	40
40	65.4	65.2
	53.4	61.7
30–50	40	62
	79	80
45	45	47
	90	73
	91	77
85–90	40	32
	43	46
90–100	64	56
97	56	37
95–100	55	29

In the earlier paper, two strains of *E. coli* were isolated from the common culture. These strains, designated as resistant and non-resistant, have now been continuously subcultured for about eight months with no noticeable change in their resistance to ultraviolet radiation. A similar separation was also made from a culture of *Staphylococcus albus*, indicating that the variation in resistivity of the various strains in a culture is not characteristic of *E. coli* only. The relative resistance to ultraviolet radiation of these two strains of *E. coli* and two strains of *Staphylococcus albus* are shown in the curves of figure 2.

To determine whether, k, of equation (2) is a constant, a pure strain of *E.*

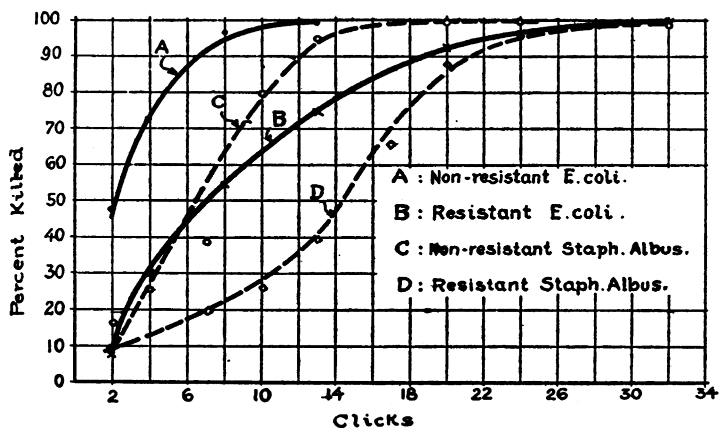

FIG. 2. PER CENT KILLED OF TWO STRAINS OF E. COLI AND STAPHYLOCOCCUS ALBUS BY DIFFERENT AMOUNTS OF ULTRAVIOLET RADIATION AS MEASURED BY THE TANTALUM PHOTOCELL ULTRAVIOLET METER

1 click = 220 microwatt seconds per sq cm of 2537A radiation.

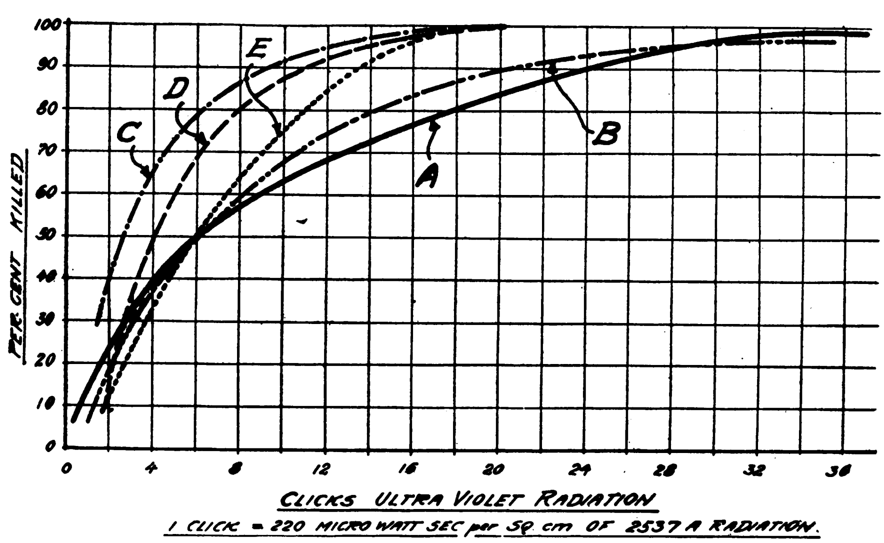

FIG. 3. THE BACTERICIDAL EFFECT OF ULTRAVIOLET RADIATION ON A PURE STRAIN OF E. COLI WHEN FIRST EXPOSED TO SUBLETHAL DOSES OF OTHER AGENTS

Curve A shows the per cent of resistant *E. coli* killed by different amounts of ultraviolet radiation. A number of Petri plates were simultaneously seeded with a resistant strain and exposed to different amounts of ultraviolet radiation in the usual manner.

Curve B is an exponential curve of the form of equation (2).

Curve C shows the effect of ultraviolet radiation following a pre-exposure to Grenz rays. A number of Petri plates were seeded with the same resistant strain of *E. coli* as for Curve A. Each of the plates was exposed to about 9600 r units of Grenz rays. The plates were then exposed to varying amounts of ultraviolet.

Curve D shows a similar effect of ultraviolet radiation following a pre-exposure (similar to C) to 17000 r units of 200 kv x-rays through a ¼-inch Pyrex glass filter.

Curve E shows the effect of pre-heating a broth culture of the same resistant strain of *E. coli* to 55°C. for 6½ minutes preceding seeding the Petri plates which were then exposed to the ultraviolet radiation.

coli was exposed to the action of a bactericidal agent and subsequently to varying amounts of ultraviolet light. The effects thus obtained for pre-exposures to different agents are shown in the curves of figure 3.

The wide divergence of curves, C, D, and, E, from the exponential curve, B, and the experimental curve, A, indicate that injury without death of bacteria due to radiation or heat is possible, thus further showing that the "single photon hit" theory does not explain the bactericidal action of ultraviolet radiation, and that the value of k, in equation (2) is not a constant.

Modified duct experiments

To obtain further quantitative data on the action of radiation on air-borne bacteria, a duct 15 feet long and 1 foot in diameter was constructed, as shown in figure 4. A diluted culture of *E. coli* was atomized into a room by the use of an Arnold Sprayer. The air was drawn from the room through a box 5 feet on the

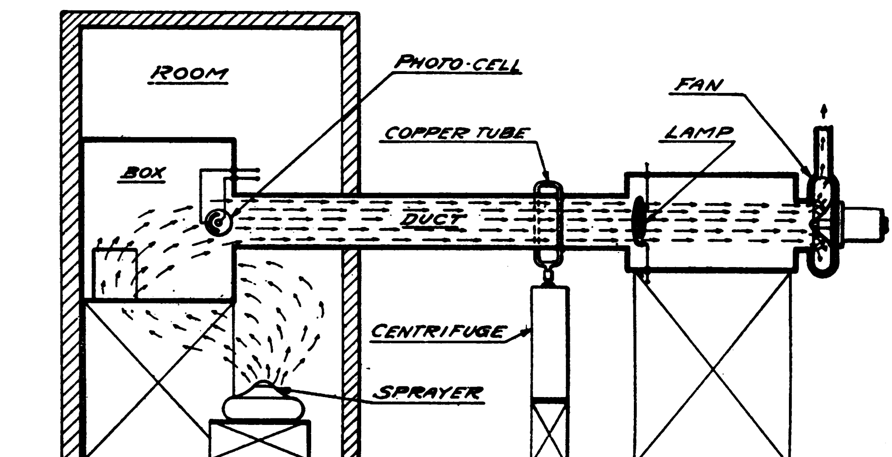

FIG. 4. DIAGRAM OF DUCT USED FOR TESTING THE BACTERICIDAL ACTION OF ULTRAVIOLET RADIATION ON AIR-BORNE ORGANISMS

side and through the duct by means of a suction fan. A spirally coiled low pressure mercury discharge lamp was placed at the exit end of the duct. Eighteen inches from the lamp a $\frac{1}{8}$ inch inside diameter copper tube, with 12 evenly spaced $\frac{1}{16}$ inch holes, was placed diametrically across the duct—similar to the arrangement used by Whisler. The air drawn through the duct was sampled by connecting a Wells air centrifuge to the two ends of the copper tube. The air was sampled alternately first with the lamp off, then with the lamp on. A number of samples were taken for different air velocities and relative humidities. In each sampling the ultraviolet intensity was measured by placing the tantalum photocell of the ultraviolet meter at the far end of the duct, thereby allowing for any variation in light intensity due to cooling of the lamp by the air currents.

Results

No difference in the bactericidal action was found at a constant velocity and for the same intensity as measured by the tantalum cell, whether a quartz or a

Corex-glass lamp was used. This shows that the short ultraviolet from the quartz lamp and the ozone produced thereby, are not material in producing an appreciable bactericidal action in the duct test.

The lethal action at constant velocity in any test was proportional to the light intensity as measured by the photocell.

For a constant intensity of ultraviolet, but varying velocity, the lethal action at a given low relative humidity was *not* proportional to the amount of radiation to which the organisms were exposed. As an illustration, in a given test at a relative humidity between 25 and 40 per cent with the full intensity of the light, 70.5 per cent of non-resistant *E. coli* were killed when the air velocity was 84

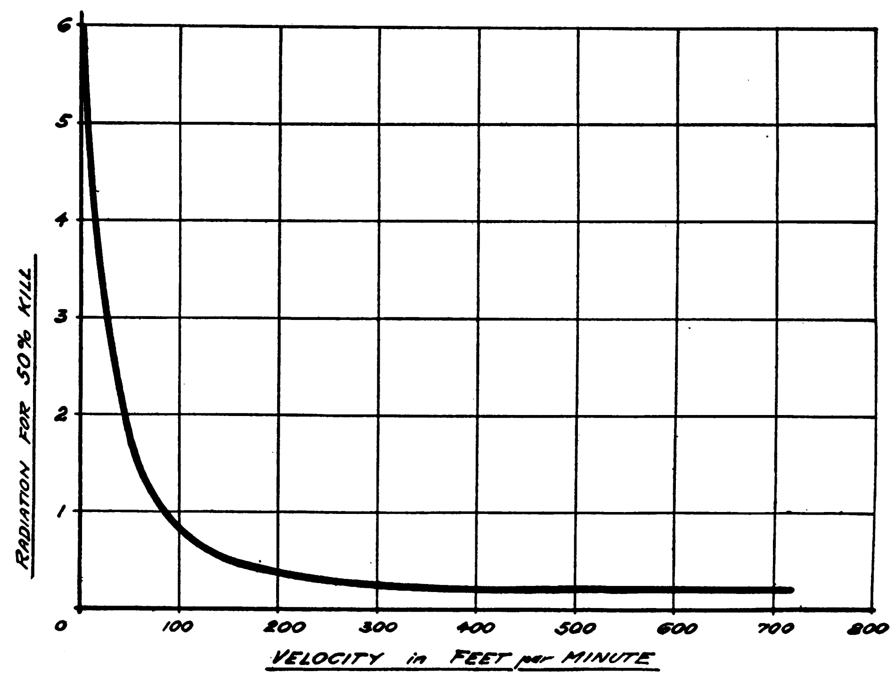

FIG. 5. RELATION BETWEEN THE AMOUNT OF ULTRAVIOLET RADIATION EXPRESSED IN ARBITRARY UNITS REQUIRED TO KILL 50 PER CENT E. COLI IN AIR AND THE VELOCITY OF THE AIR MOVEMENT IN THE DUCT

ft. per min.; while at 353 ft. per min. the added velocity cooled the lamp and reduced the intensity of the ultraviolet by 15 per cent, but resulted in a reduction of the lethal action to only 52.5 per cent. Thus, the amount of exposure was reduced to about one-fifth, with a reduction in lethal action of only eighteen per cent (from 70.5 to 52.5 per cent), indicating a greater killing action for the higher velocity. This same general phenomenon was noticed about two years ago, but was dismissed at that time as being ridiculous. The relation between the radiation in arbitrary units required to kill 50 per cent of the bacteria and the velocity of air flow observed at that time is shown by the curve of figure 5.

A series of tests was outlined to determine the cause of these peculiar results. On three separate days a number of tests were made in the duct with a non-resistant strain of *E. coli* at a velocity of 350 ft. per min. and with the full light intensity. A similar number of tests was made, but for an air flow reduced to one-fourth or one-fifth this velocity, and with the light intensity reduced in the same ratio, so that the bacteria in both experiments were exposed to the same amount of radiation. Two similar runs were made on other days using a resistant strain of *E. coli*, and finally three runs were made with non-resistant *E. coli* at high relative humidity. The average bactericidal action for each of these tests is shown in table 2.

TABLE 2

Effect of air velocity upon the bactericidal action for the same amount of ultraviolet radiation

STRAIN OF E. COLI	VELOCITY OF AIR	PER CENT KILLED	RELATIVE HUMIDITY
	ft./min.		%
Non-resistant	353 84	90.8 67.4	30–40
Non-resistant	353 84	84 70.8	45
Non-resistant	350 70	78.5 29.7	35
Resistant	350 84	80.3 43.5	25–40
Resistant	350 70	81.7 39	35
Non-resistant	350 70	32.3 31.5	90–95
Non-resistant	350 66	64 51	85–96
Non-resistant	353 84	47 45.5	80–90

The following conclusions are derived from table 2.

1. At low relative humidity, the per cent bacteria killed, as determined by the use of the centrifuge for sampling, is definitely greater at high air velocity than at low velocity for both resistant and non-resistant strains of *E. coli.*

2. At high relative humidity, there is no detectable difference in the bactericidal action at high and low velocities.

3. At low humidity and high velocity, the lethal action on the non-resistant strain is not materially greater than on the resistant strain.

4. Since, roughly, the same light intensity as recorded by the photocell was used at the high velocity for both the high and low humidity tests, the radiation

appears to be more effective at low relative humidity when samplings are made with the *centrifuge.*

DISCUSSION

In the tests of Whisler, approximately $\frac{1}{3}$ of a cubic foot of air per min. was taken with an air centrifuge from the duct through twelve $\frac{1}{16}$ inch diameter holes in a $\frac{1}{8}$ inch copper tube placed across the duct. This corresponds to an average velocity entering the $\frac{1}{16}$ inch holes of between 1300 and 1400 feet per minute. In sampling the air in the duct under such conditions, it is evident that the air and particles floating in the air of the duct are deflected from their normal course on entering the copper tube. Lighter particles are more readily deflected than the heavier ones so that the sampling is selective. This selective action is more marked for high velocity air flow through the duct than for low velocities. Evidently, therefore, sampling of the bacteria from an air stream in a duct at low relative humidity using an air centrifuge is selective, if all the bacteria are not identically distributed (that is if, k, is not a constant). At high relative humidity the bacteria are associated with water droplets and this selectivity becomes negligible. Here the size of the droplet determines the size of the particles associated with the bacteria.

From these tests it appears that the seemingly greater resistivity of air-borne bacteria to ultraviolet radiation at high relative humidity previously reported is due to the selectivity of the sampling device and not to an inherently greater resistivity of the organism. Phelps and Buchbinder (1941) report a still different type of selectivity in air samplings taken with the air centrifuge.

By measuring the light intensities at different points along the duct, the calculated amount of radiation a bacterium is exposed to in the tests of table 2 indicates an exposure of approximately (2 clicks) 440 microwatt seconds per sq. cm. of 2537A radiation. This quantity of radiation is approximately the amount by which a noticeable percentage of both resistant and non-resistant *E. coli* is killed on the agar of Petri plates, as reported in the previous paper and as seen from the curves of figure 2. Consequently, with a selective method, such as the centrifuge for sampling the bacterial contamination of the air at low humidity, the apparent lethal radiation required is of the same order of magnitude as that necessary to destroy the less resistant organisms in a culture seeded on the agar of a Petri plate.

The true lethal power of ultraviolet radiation on air-borne bacteria can be experimentally measured only with a non-selective method of air sampling. Since the radiation found necessary in the duct for killing air-borne bacteria is of the same order of magnitude as that required for the more sensitive organisms in a culture on agar, it appears that the discrepancy between the duct and tower experiments is due entirely to the selective sampling of the test apparatus used in the duct experiments, and that an air-borne bacterium is not ten to one hundred times as sensitive to radiation as is the same bacterium on a Petri plate. From the tower experiments the conclusion is that the lethal radiation for an air-borne bacterium is the same as for the same bacterium on agar.

SUMMARY

By the use of a specially devised tower experiment, the sensitivity to ultraviolet radiation of air-borne bacteria and of similar organisms on the surface of agar was shown to be the same.

Experiments are described to show that bacteria may be injured by heat, Grenz rays or x-rays so that the surviving organisms are less resistant to ultraviolet radiation. This is incompatible with the "single photon hit" theory.

All the bacteria in a pure strain of a culture are not identical and the term, k, in the exponential equation generally used for expressing the survival ratio for different exposures to ultraviolet radiation, is not a constant as is commonly assumed.

The air centrifuge, as used in determining the bacterial contamination of air moving through a duct at low humidity, is selective in taking the bacteria from the air. This appears to explain the reason for the general statements that air-borne bacteria at low humidity are more vulnerable to ultraviolet radiation than when on the surface of agar, and that at low relative humidity the bactericidal action is greater than for high humidity.

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